Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Short Communication

The effect of rFVIIa on pro- and anti-inflamatory cytokines in serum and cerebrospinal fluid in a swine model of traumatic brain injury ☆

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ARTICLE INFO

ABSTRACT

Traumatic brain injury (TBI) is associated with significant infectious and inflammatory complications. Though increasing evidence suggests that rFVIIa administration may be efficacious for the pre-hospital treatment of TBI, the FVIIa-tissue factor complex has been shown to be immunologically active. To date the cytokine response to rFVIIa administration for the treatment of TBI has not been evaluated.

Twenty anesthetized immature Yorkshire swine underwent fluid percussion TBI. At 15 min following injury, animals were randomized to receive either 90 µg/kg rFVIIa (rFVIIa) or nothing. Animals were observed for 6 h and then euthanized. Plasma and cerebrospinal (CSF) samples were collected at 0 min and 360 min, and ELISA analysis of TNF-α, IL-1β and IL-10 was performed. Survival in both groups was 100%. Baseline cytokine concentrations were not statistically different between rFVIIa and control animals in plasma or CSF. Animals in both groups did not have significant changes in plasma cytokine concentrations following TBI. Control animals did not demonstrate significant changes from baseline of CSF cytokine concentrations following TBI. The administration of rFVIIa however, resulted in significant increases in CSF TNF-α concentration (232.0 pg/ml ± 75.9 vs 36.4 pg/ml ± 10.4, $p = 0.036$) and IL-10 concentration (10.7 pg/ml ± 0.6 vs 8.8 pg/ml ± 0.1, $p = 0.015$). IL-1β concentrations were not significantly changed over the experimental time course. These results suggest that rFVIIa administration for the treatment of TBI is not immunologically inert, and is associated with increased CSF concentrations of TNF-α and IL-10.

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1. Introduction

Recombinant Factor VIIa (rFVIIa) is approved for use in the treatment and prophylaxis of bleeding in patients with Factor VIII and IX deficiencies. However, off-label use of rFVIIa has demonstrated efficacy in the treatment of severe bleeding associated with trauma and massive transfusions [1,2]. rFVIIa has also been utilized to reverse both therapeutic and acquired coagulopathies and for the treatment of bleeding in traumatically brain injured patients [3]. In all of these trials, rFVIIa was administered only in the hospital setting, after identification of either clinically relevant bleeding

or coagulopathy. Moreover, there was large variability in the timing of administration from within 2 h of admission to as late as 37 days post admission [2,4]. Recently, the results of animal studies utilizing early, pre-hospital, administration of rFVIIa after traumatic brain injury (TBI) have been published. This study demonstrated a reduction in hemorrhage volume, and attenuated neurodengeneration suggesting a possible role for rFVIIa in the immediate management of TBI [5]. To date, however, no study has evaluated the effect of rFVIIa on the inflammatory milieu following TBI. The acute post injury phase in TBI patients is complicated by significant risk of infectious and inflammatory complications [6]. The potential benefits of hemorrhage volume reduction and decreased neuron degeneration provided by rFVIIa have not been investigated. Moreover, such possible benefits must be weighed against the fact that the FVIIa-tissue factor complex is immunogenic [7].

In this current study, we measured levels of inflammatory and anti-inflammatory cytokines in the serum and cerebral spinal fluid (CSF) of swine subjected to fluid percussion TBI who received either rFVIIa or nothing 15 min after injury. This study utilizes

Report Documentation Page

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1. REPORT DATE **DEC 2010**	2. REPORT TYPE	3. DATES COVERED **00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE **The effect of rFVIIa on pro- and anti-inflamatory cytokines in serum and cerebrospinal fluid in a swine model of traumatic brain injury**	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Naval Medical Research Center,Operational and Undersea Medicine Directorate,503 Robert Grant Avenue,Silver Spring,MD,20910**	8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Traumatic brain injury (TBI) is associated with significant infectious and inflammatory complications. Though increasing evidence suggests that rFVIIa administration may be efficacious for the pre-hospital treatment of TBI, the FVIIa-tissue factor complex has been shown to be immunologically active. To date the cytokine response to rFVlla administration for the treatment of TBI has not been evaluated. Twenty anesthetized immature Yorkshire swine underwent fluid percussion TBI. At 15 min following injury, animals were randomized to receive either 90 jlg/kg rFVIIa (rFVlla) or nothing. Animals were obseJVed for 6 h and then euthanized. Plasma and cerebrospinal (CSF) samples were collected at 0 min and 360 min, and ELISA analysis of TN F-a, IL-l p and ll-1 0 was performed. SuJVival in both groups was 100%. Baseline cytokine concentrations were not statistically different between rFVlla and control animals in plasma or CSF. Animals in both groups did not have significant changes in plasma cytokine concentrations following TBI. Control animals did not demonstrate significant changes from baseline of CSF cytokine concentrations following TBI. The administration of rFVIIa however, resulted in significant increases in CSF TNF-a concentration (23 2.0 pgjml ? 75.9 vs 36.4 pgjml ? 10.4, p = 0.036) and lL-10 concentration (10.7 pgjml ? 0.6 vs 8.8 pg/ml ? 0.1, p = 0.015). ll-1 p concentrations were not significantly changed over the experimental time course. These results suggest that rFVIIa administration for the treatment of TBI is not immunologically inert, and is associated with increased CSF concentrations of TNF-a and Il-10.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**4**	

samples from a pilot study investigating the pre-hospital role of rFVIIa and represents the first study of inflammatory cytokinetics following rFVIIa administration.

2. Materials and methods

2.1. Surgical procedure

Swine experiments were conducted according to principals of the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care Committee; all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International.

Twenty male and female immature Yorkshire swine were fasted 12 h preceding surgical procedures with water ad libitum. Anesthesia was induced with intramuscular ketamine (30 mg/kg) and atropine sulfate (0.05 mg/kg), and maintained with inhaled isoflorane. Animals were endotracheally intubated and placed prone on the operating table. Isouflorane was maintained at a mean alveolar concentration of 1.5–2% in medical air. A scalp flap was elevated and removed using electrocautery. A 16 mm craniotomy was created using a handheld drill in the right parietal region adjacent to the sagittal suture and anterior to the coronal suture. A T-shaped bolt was secured in the craniotomy abutting intact dura. The bolt was then coupled to a fluid percussion hammer and pendulum. Four additional smaller craniotomies were necessitated for placement of two oxygen monitor probes (Licox MCB, Integra Life Sciences, Plainsboro, NJ) in the bilateral frontal cortices, a sagittal sinus catheter for venous blood sampling in the posterior sagittal suture and a neonatal intraventricular catheter (Integra Neurosciences, Plainsboro, NJ) was placed through a craniotomy overlying the left lateral ventricle for intracranial pressure (ICP) monitoring and CSF sampling. An arterial catheter was placed via carotid cut-down, and a 7.5-French (Fr) pulmonary arterial catheter (Edwards Lifesciences, Irvine, CA) was inserted through a 9-Fr sheath introducer in the contralateral jugular vein. Normal saline was provided at a maintenance rate of 1.5–2 cc/kg/h.

At 0 min, the pendulum was released, striking a plexiglass piston transmitting a 15-ms pressure pulse to the dura and measured via parallel oscilloscope. Injury force was between 2.5 and 3 atmospheres of pressure. At 15 min pigs were randomized to receive a bolus of either 90 μg/kg rFVIIa (NovoSeven, NovoNordisk, Bagsvaerd, Denmark) in sterile water (rFVIIa, $n = 10$) or nothing (control, $n = 10$).

Hospital arrival was simulated at 60 min. At this time, neuromuscular blockade was provided via intravenous vecuronium, and mechanical ventilation was initiated. FIO_2 was titrated to maintain a transcutaneous O_2 saturation above 92%. Starting at hospital arrival, up to two boluses of mannitol were infused as needed for intracranial hypertension greater than 20 mm Hg. Animals were observed and euthanized at 360 min.

2.2. Cytokine analysis

Blood was collected at 0 min, prior to TBI, and 360 min, just prior to euthanasia. Whole blood was treated with EDTA and centrifuged at 2500 rpm for 10 min at room temperature. Plasma was isolated and stored at −80 °C. CSF was collected from the intraventricular catheter at 0 min and 360 min and stored at −80 °C. Cytokine concentrations were quantified using enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-1β and IL-10 (Bio-

Source International, Camarillo, CA) in duplicate according to the manufacturer's protocols.

2.3. Statistical analysis

Statistical analysis was performed using two-tailed t-tests assuming unequal variances and significance was determined when $p < 0.05$.

3. Results

Survival to experimental endpoint in both groups was 100%. Physiological parameters including heart rate, blood pressure and ICP did not vary significantly throughout the experiment. All cytokine concentrations in both plasma and CSF samples at 0 min did not differ between rFVIIa and control groups ($p > 0.05$).

TNF-α concentration in plasma at 6 h following TBI in control animals did not change from baseline (81.7 pg/ml ± 18.2 vs 69.0 pg/ml ± 6.5, respectively, $p = 0.53$). The administration of rFVIIa following TBI did not alter serum TNF-α concentration from baseline (71.3 pg/ml ± 8.6 vs 74.0 pg/ml ± 8.9 at 0 min and 360 min, respectively, $p = 0.86$). CSF concentration of TNF-α did not change significantly in the control group from baseline to experimental endpoint (60.3 pg/ml ± 27.4 vs 83.1 pg/ml ± 24.0, $p = 0.55$). Early administration of rFVIIa after TBI, however, was associated with a 4.8-fold increase in CSF TNF-α (36.4 pg/ml ± 10.4 vs 232.0 pg/ml ± 75.9, $p = 0.036$). (Fig. 1A).

IL-1β concentration did not change from baseline in either plasma or CSF following TBI in the control group (plasma: 23.9 pg/ml ± 2.1 vs 37.3 pg/ml ± 9.8, $p = 0.22$. CSF: 446.89 pg/ml ± 24.7 vs 420.39 pg/ml ± 29.1, $p = 0.51$). The administration of rFVIIa was not associated with changes over baseline at 360 min in either plasma or CSF concentrations of IL-1β (plasma: 27.5 pg/ml ± 9.0 vs 46.6 pg/ml ± 13.5, $p = 0.26$. CSF: 366.8 pg/ml ± 36.7 vs 430.7 pg/ml ± 43.5, $p = 0.28$.). (Fig. 1B).

Post TBI, IL-10 concentrations were not different from baseline in either serum or CSF in the control group (Plasma: 17.8 pg/ml ± 2.7 vs 17.7 pg/ml ± 2.7, $p = 0.98$. CSF: 9.0 pg/ml ± 0.2 vs 9.1 pg/ml ± 0.1, $p = 0.65$). While plasma IL-10 concentrations were not statistically different from baseline at 6 h following TBI in rFVIIa animals (16.6 pg/ml ± 0.3 vs 16.5 pg/ml ± 0.4, $p = 0.82$), CSF concentration of IL-10 increased significantly from 0 min to 360 min in rFVIIa animals (8.8 pg/ml ± 0.1vs 10.7 pg/ml ± 0.6, $p = 0.015$). (Fig. 1C).

4. Discussion

Brain injury and hemorrhage are the first and second most common causes of mortality following trauma and are more common than all other causes of death combined [8]. Numerous studies have reported rFVIIa's efficacy in treating traumatic bleeding and reversal of both iatrogenic and acquired coagulopathies following TBI. A large phase III clinical trial evaluating the effect of rFVIIa administration in patients with CT confirmed spontaneous intracerebral hemorrhage (the FAST trial) found that a 80 μg/kg rFVIIa bolus significantly reduced hematoma expansion, but failed to improve 30 day mortality [9]. The failures of recent studies to definitively determine the efficacy of rFVIIa administration following TBI may stem, in part, from the large variability in timing of administration and dosage.

Recently, Zhang and colleagues studied the effect of rFVIIa in the "pre-hospital" setting. Anesthetized swine underwent fluid percussion TBI, followed by a 720 μg/kg rFVIIa bolus at 5 min post injury, before the identification of coagulopathy or clinically signif-
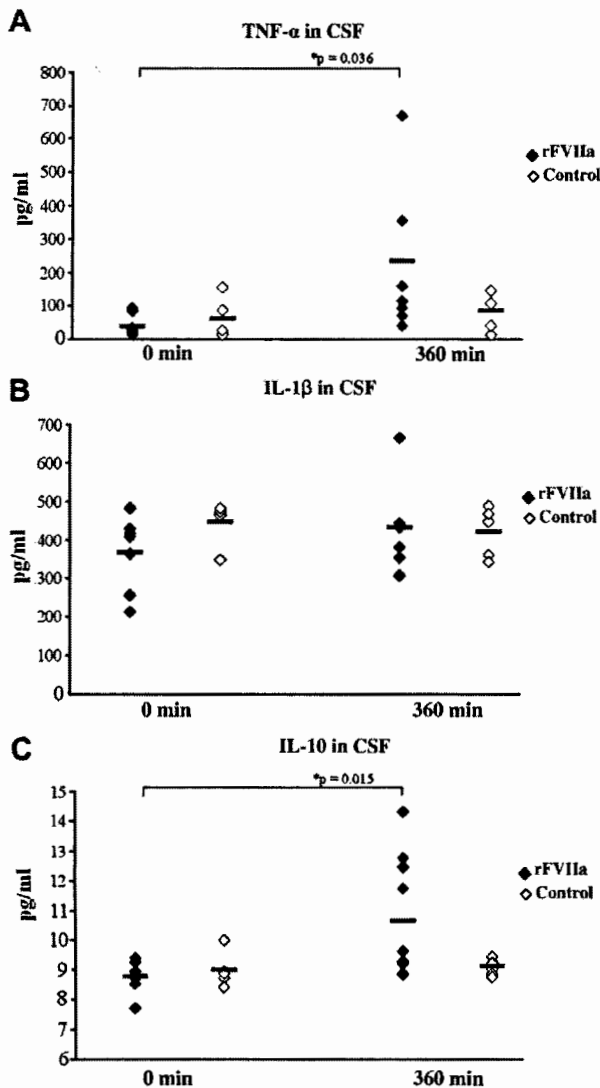
Fig. 1. Concentration of TNF-α (A), IL-1β (B) and IL-10 (C) in CSF at 0 min and 360 min. The CSF concentrations of TNF-α and IL-10 increase significantly 6 h following TBI in animals who received rFVIIa ($p = 0.036$ and 0.015, respectively). CSF cytokine concentrations are not significantly different before and 6 h after TBI in control animals.

icant bleeding. They found a significant reduction in hemorrhagic contusion volume and decreased neural degeneration [5].

The administration of rFVIIa may have complications. The FAST investigators reported a 125% relative risk increase for serious arterial thromboembolic events. However, the administration of rFVIIa for trauma did not cause increased thromboembolic events in the largest randomized placebo controlled study of rFVIIa in blunt and penetrating trauma [10].

Our study is the first to examine the effect of rFVIIa administration for the treatment of TBI on cytokine flux. Numerous experiments have demonstrated that the activated Factor VII–tissue factor (TF) complex is immunogenic. The administration of rFVIIa produced elevated concentrations of circulating IL-6 and 8 in healthy volunteers [11] and blocking the FVII-TF complex attenuated the IL-10 response to endotoxin stimulated human subjects [12]. These results suggest that activated FVII may play an important role in the inflammatory cascades that accompany septic shock.

Given the significant exposure of previously sequestered proteins, and activation of the coagulation cascade, similar inflamma-

tory pathways are relevant following brain trauma. TBI is an independent risk factor for infectious and inflammatory complications following traumatic injury. Patients who present with a Glasgow Coma Score (GCS) of eight or less, have a 41% chance of developing pneumonia, 29% chance of developing significant hypotension, and a 10% chance of developing septicemia. [6] While the rate of ventilator associated pneumonia (VAP) has been estimated at between 8% and 28% in non-trauma patients, intubated patients with TBI have been shown to suffer VAP at a rate of up to 60%. [13]

The pro-inflammatory cytokine TNF-α and the anti-inflammatory IL-10 are both elevated in human CSF following TBI. In experimental studies, TNF-α neutralization has resulted decreased brain edema, blood–brain barrier dysfunction and neurodegeneration [14]. While IL-10 administration improved neurologic outcomes in traumatic brain injured rodents [15], elevated levels of CSF IL-10 following TBI independently associated with mortality in children [16]. Our data suggests that rFVIIa administration following fluid percussion TBI increases CSF concentrations of both of TNF-α and IL-10. The clinical significance of this finding remains to be realized. And whether it is of consequence compared to the benefit of reduced hemorrhage volume and decreased neurodegeneration imparted by rFVIIa administration is unknown.

Perhaps the most striking finding in this experiment is that in this model of fluid percussion TBI, animals who did not receive rFVIIa did not demonstrate any perturbations from baseline in either plasma or CSF concentrations of any of the measured cytokines. Multiple rodent studies of fluid percussion TBI showed significant early elevations of IL-1β and TNF-α transcription and translation in the brain. Rat models have demonstrated increases in TNF-α, IL-1β and IL-10 mRNA and protein concentrations as 1 h post TBI [17,18]. However these samples were derived from homogenized brain sections, and the cytokines may represent mononuclear cells that were present in the cerebral vasculature as a response to injury, but sequestered from the neurons and CSF by an intact blood brain barrier. Given the exceedingly small volume of rodent CSF, fluid analysis would be prohibitively difficult. To date, the effect of fluid percussion TBI on swine cytokine profiles has not been measured. It is possible that although fluid percussion causes gross and histopathological changes in injured cortices, the injury severity is not sufficient to produce a brisk inflammatory response.

5. Conclusion

rFVIIa is not immunologically inert. In a fluid percussion model of TBI in swine, pre-hospital rFVIIa administration was associated with increased in CSF concentrations of TNF-α and IL-10. Consequently, rFVIIa may alter the inflammatory sequelea following TBI. The clinical impact of this involvement remains to be investigated in future studies.

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